Characterization of Hop Extracts and Extracts from some Selected Nigerian Plants by GC-MS and Brewing Qualities Analyses

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ABSTRACT

Humuluslupulus L (hop) plants and four selected Nigerian plants namely *Azadirachtaindica*(neem), *Garcinia kola* (bitter cola), *Gongronemalatifolium*(heckel) and *Vernoniaamygdalina* (bitter leaf) as substitutes for hops in beer brewing were characterized in terms ofmethanolic and aqueousextracts using Gas Chromatography - Mass Spectrometer (GC-MS-Q2010 plus, Shimadzu, Japan). The brewing qualities (iso-alpha acid and essential oil contents)of the extracts were investigated using standard methods. Statistical analysis was carried out on the result of brewing qualities tests by the application of one way analysis of variance (ANOVA). The GCMS results showed that these plants contained metabolites comparable to those of hops, although some metabolites[dehydro-cohumulunic acid; 4,4-dimethyl-2-buten-4-olide; 1,2-dimethyl-cyclopropane carboxylic acid; lupulone; 2,5-dimethyl-2-hexanol; 4,4,5,5-tetramethyl-bicyclo hexyl-6-ene-2,3-dione; octadecanoic acid, oxiranyl methyl ester and 1,2-benzenedicarboxylic, bis(-2-ethyl hexyl) ester] present in hops were absent in the Nigerian plants. Isomerized hop, hop leaf, *G. kola* and *V. amygdalina*contained a total number of 14, 11, 12 and 9 metabolites respectively while *A. indca*and *G. latifolium* each contained a total number of 10 metabolites.The brewing qualities investigated showed that iso-alpha acid ranged from 8.78-12.53ppm and essential oil ranged between 5.44-10.12g/100g. This study revealed that the brewing qualities of all the extracts did not differ significantly. Hence, extracts from tested Nigerian plants present potential substitutes for hops in the Nigerian brewing industry without significant alteration to the brewing qualities. Consequently, academic activity in the area of mixtures/blends of extract of plant species which mimic hop taste is strongly recommended.

Key words: hops, extract, GC-MS, metabolites, Nigerian plants, substitutes, bitterness.

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INTRODUCTION

*Humulus lupulus*L. (hop) is a perennial dioecious climbing plant of hemp (*cannabis)* family and belonging to the order (*urticales*) which also includes the nettle family (Kunze, 1999). Only two species of *Humulus* are recognized: *Humulus lupulus L.* (*H. americanus, H. neomexicanius* and *H. cordifolius)* and *H. japonicas* sieb. The latter is an annual ornamental climbing plant from Japan devoid of resin and therefore of no brewing value. The genus *Humulus*is included in the natural family *Cannabinaceae* together with *cannabis,* which is only represented by *C. sativa* (Indian hemp, marihuana or hashish). Chemical similarities are seen between *H. lupulus* and *C. sativa* but the resins of the two species are completely distinct. Those of the hop provide the bitter principles of beer whilst those of the *cannabis* include the psychotomimetic principles of drug (Crombie and Crombie, 1975).From medieval times, herbs have been used to flavour and preserve fermented malt liquors but only female hop inflorescence is used on a commercial scale today. The hop plant is grown in the temperate regions of the world, solely to meet the demands of the brewing industry (Hough, *etal*., 1982). The brewing value of the hop is found in its resins and essential oils.

Garcinia kola (bitter cola), an angiospermae, belonging to the family *Guttiferae*, is known in commerce as bitter cola. Bitter cola is a highly valued ingredient in African ethno-medicine because of its varied and numerous uses which are social and medicinal; thus making the plant an essential ingredient in folk medicine, and medicine plants such as *G. kola* are found to be an important source of new chemical substances with potential therapeutic benefits (Eisner, 1990; Iwu, 1990).

Azadirachta (neem) is a genus of two species of trees in the Mahogany family, *Meliaceae*. Numerous species have been proposed for the genus but only two are currently recognized, *Azadirachta excelsa* and the more economically important tree,

Azadirachta indica which is the only species in Nigeria (Keay*etal*., 1964). Products made from neem are found to be antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative (Buttler and Bailey, 1973; Mabberley, 1995).

Vernonia amygdalina biter leaf) is a shrub or small tree with petiolate leaf of about 6mm in diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste (Igile *et al*., 1995).They are used as vegetable and to stimulate the digestive system, as well as reduce fever (Onwuka *e tal*., 1989) and as local medium against leech which transmits bilharziasis (Fayemi, 1982).

Gonogronema latifolium (heckle) is a climbing shrub of the family *Asclepiadeceae*. It is known as *utazi* in many Nigerian languages. It grows up to 5m long, stems hollow, all parts soft-hairy to glaborous, with woody base and fleshy roots, containing latex. Leaves opposite, simple and entire; petiole up to 2.5 -3cm long. Fruit, a pair of pendent follicles, each one narrowly cylindrical, 7 -10cm x 1 -1.5cm, yellow and many seeded (Keay *et al*., 1964). Different leaf extracts showed moderate to promising antioxidant, anti-inflammatory, hepatoprotective, anti-plasmodial, anti-asthmatic, anti-sickling, anti-ulcer, analgesic, antipyretic, gastrointestinal relaxing, laxative and stomachic activities (Dike, 2010; Emeka and Obiora, 2009; Nwanjo and Alumanah, 2006; Okolie *et al*., 2008; Oliver-Bever, 1986).

In Nigeria, hops are imported, and since beer production in Nigeria has increased recently due to ready markets, the importation of hops becomes inevitable. Thus, huge amounts of foreign exchange are being spent by this sector in importation, hence, the urgent need to investigate some potential Nigerian plants that can substitute hops in the Nigerian beer industry. This study takes into consideration other competitive uses of these selected Nigerian plants.

Varietal characterization of hop (*Humulus lupulus L*) by GC-MS analysis of hop cone extract has been chronicled by Shellie and co-workers (Shellie *etal*., 2009). The use of

bitter leaf (*Vernonia amygdalina*) as local substitute for hops in the Nigerian brewing industry had been investigated by Adama and others (Adama *et al*., 2011).

Materials and Methods

Procurement of Materials

Hop leaf and isomerised hop extract were respectively purchased from Youngs Ubrew Goldings Hops and Ritchies both in the United Kingdom. The leaves of *A. indica, G. latifolium, V. amygdalina* and the seeds of *G. kola* were obtained from the herbarium of Nnamdi Azikiwe University, Awka. Chemicals used were as detailed by AOAC, ASBC, and IOB.

Sample Preparation

Except for the isomerised hop extract prepared by Ritchies, each plant sample was milled and vacuum dried at 50^{o}C. Two kilograms (2kg) of each plant material thus prepared was stored in a dessicator for the rest of the experiment. Three hundred grams (300g) each of the resulting powders were then used to obtain the methanolic extracts by steeping procedure.

Methanol Extraction

The methanol extract was prepared by steeping 300g of the dry powdered plant material in 1.5 litres of methanol at room temperature in a tight fitting round bottom flask for forty eight hours. The mixture was filtered first through a Whatman filter paper (No. 42) and then through a sintered glass funnel. The filtrate was concentrated using a rotary evaporator with water bath set at 40^{o}C for 2 hours to obtain each extract. The extract was stored in amber coloured reagent polypropylene bottle in a deep freezer (Thermofrost, Mod.TR150S) at -5^{o}C for subsequent analysis.

GC-MS Technique

GCMS analysis was performed using a Shimadzu GCMS-QP2010 plus (Schimadzu Oceania, Japan). A 60m x 0.25mm id BPX – 35 capillary columns with 0.25µm film thickness was used. Helium was used as carrier gas at a head pressure of 241250Pa to provide an initial flow rate of 2ml/min. A 1µl spitless injection (230^{o}C, 1.5min) was used. The GC temperature gradient was 85^{o}C to 330^{o}C at the rate of 4^{o}C/min and held at 330^{o}C for 10 minutes. Full-scan mass spectra were collected from 85 to 550 mass/charge ratio at a data acquisition rate of10 spectra/second. The MS transfer line was held at 250^{o}C and the ion source temperature was 200^{o}C.

Deconvolution of metabolites

GC-TOFMS is a benchmark approach for metabolomics data acquisition (Fernie and Shauer, 2008) from chromatographic peaks. The GC component provides excellent sensitivity and sufficiently high data density to permit the deconvolution of overlapping metabolite peaks. It thus exhibits the power of clearly differentiating two or more closely associated chromatographic peaks which are commonly found in metabolite chromatograms. In addition, the MS component displays capacity to analyse each eluted chromatographic peak and subject the mass spectra to comparative analysis using a well appointed metabolite library of simulated mass spectral information (Finar, 1975; Christian, 2004). In the present investigation, a scanning mass spectrometer was used to obtain chromatograms for the samples. Spectrum matching is achieved by programming the soft ware to compare the chromatogram of the mass spectra to simulated library peaks.

Determination of Iso-Alpha Acid

Iso- alpha acid was determined according to ASBC. Ten mililitre of the extracts was measured into a 50ml centrifuge tube. To this was added 1ml 3M hydrochloric acid and 20ml iso-octane. The tube was stoppered and agitated for 15 minutes. The tube was further centrifuged for 3 minutes at 3000 rpm.An iso-octane blank was prepared

into a 1cm quartz curvet. A clear iso-octane phase in the centrifuge tube was pipetted into another curvet and stoppered. The spectrophotometer's λ was set at 275nanometer and zeroed with the blank before the absorbance of the sample was read. The reading was multiplied by 50 and the result expressed as:

Absorbance at 275 nanometer x 50 = Bitterness in IBU, International Bitterness Unit where 1IBU is equivalent to 1ppm of iso-alpha acid.

Essential Oil Determination

Steam distillation method as adopted by Adama *et al*. was employed. Twenty (20) grams of the ground sample and 1500ml of water for steam generation were used at moderate heating rate. 1500ml of water was introduced into the bottom chamber of the still. The chamber was covered with a perforated metal plate in which a white filter cloth was placed. 20g of the ground sample was then placed on the filter cloth. This was further covered with white filter cloth. The last perforated metal plate was placed on the top compartment. Finally, the still was made air-tight with the last covering to prevent the escape of the steam-oil mixture during heating. The set-up was then connected to a condenser via a pipe fixed at the top of the extraction still where an opening had been made. The delivery tube from the condenser was connected to the separating funnel to receive the mixture of steam and oil on condensation. The set-up was then mounted and connected to the heating source for extraction time of 120 minutes. At the end of the time interval, the set-up was switched off and allowed to cool. The water-oil mixture was decanted to separate the oil from the water at the water-oil interface. Thereafter, the mass of the sample after extraction and drying in an electric oven was collected in a sample bottle and its mass recorded. This procedure was repeated several times to obtain sufficient quantity of the essential.

Statistical Analysis

In the test of significant difference, One Way Analysis of Variance (ANOVA) is the most suitable tool as it has the capacity to show the existence of difference at 5% level

of significance (Gupta, 2011). In ANOVA, two hypotheses, H_0 and H_1 are stated and tested for:

H_0; there is no significant difference among samples of interest.

H_1; there is significant difference among samples of interest.

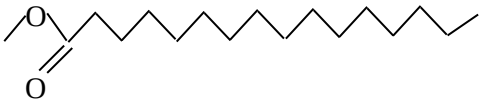
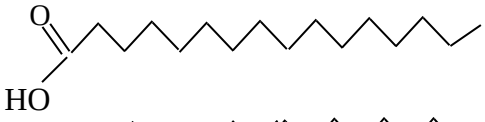
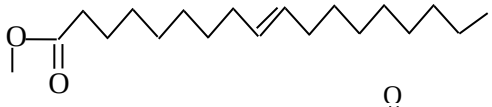
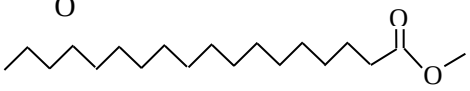
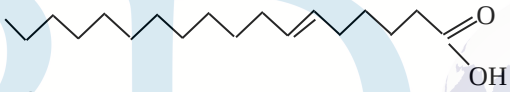
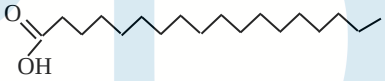
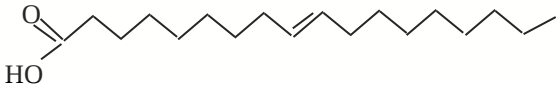
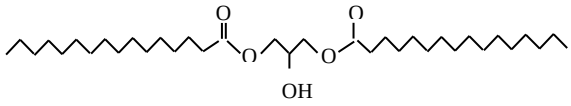
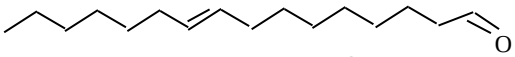
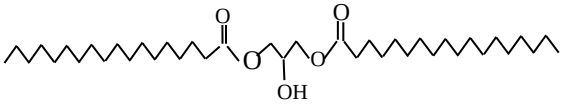
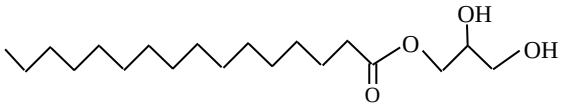
The result of the p- value (significance value) is used to accept or reject either of the hypotheses.

RESULTS AND DISCUSSIONS

Garcinia kola

The extract of *G. kola* contained twelve metabolites and these metabolites are shown in Table 1. It is shown in the Table that 6-octadecenoic acid was highest with a proportion of 44.09 %.

Table 1: Relative Proportion of Metabolites of *G. kola*

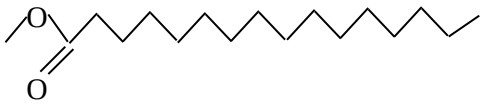
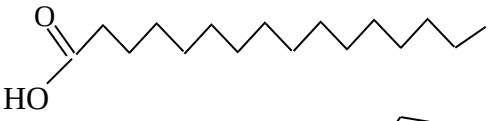
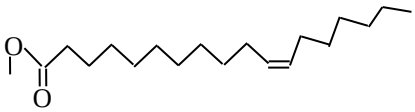
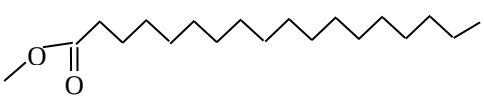
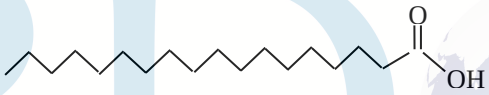
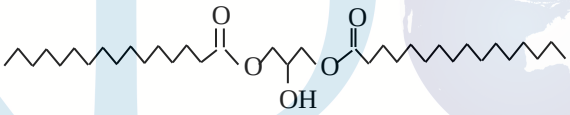
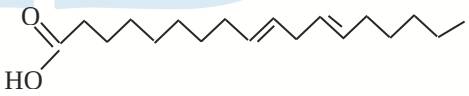
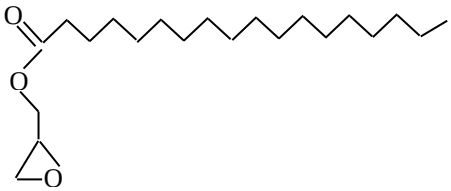
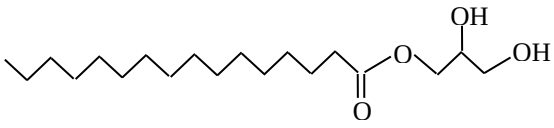
S/N	Metabolite	Formula	Structure	Relative Proportion (%)
1.	Hexadecanoic, methyl ester	$C_{17}H_{34}O_2$		0.69
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$		9.30
3.	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		2.84
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$		1.62
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$		44.09
6.	Octadecanoic acid	$C_{18}H_{36}O_2$		23.31
7.	2-Methyl-3, 13-octadecadein-1-ol	$C_{19}H_{36}O$		4.23
8.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$		1.04
9.	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{35}H_{68}O_5$		1.92
10.	9-Hexadecenal	$C_{16}H_{30}O$		7.07
11.	Octadecanoic acid, 2-hydroxyl-1, 3-propanediyl ester	$C_{39}H_{76}O_5$		2.84
12.	Hexadecanoic acid, 2, 3-dihydroxypropyl ester	$C_{19}H_{38}O_4$		1.04

There was also presence of 9, 12-octadecadienoic acid, the grape seed oil which is an essential oil of the hops. Hexadecanoic acid was least in proportion with 0.69 %.

Azadirachta indica

A. indica extract contained ten (10) metabolites. Table 2 shows these metabolites and their relative proportion. It is shown in the Table that 6-octadecenoic acid had the highest proportion of 44.96 % and hexadecanoic acid- 2, 3-dihydroxypropyl ester had the least proportion of 0.85 %.

Table 2: Relative Proportion of metabolites of *A. indica*

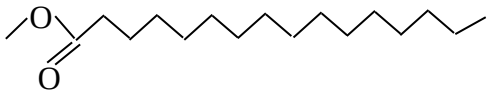
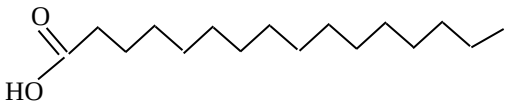
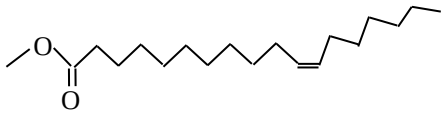
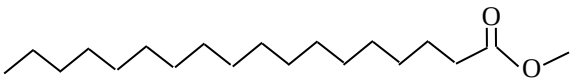
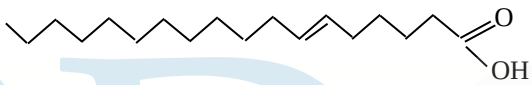
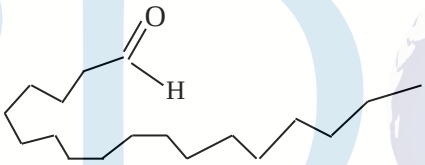
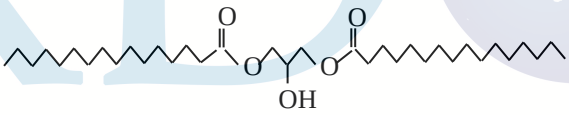
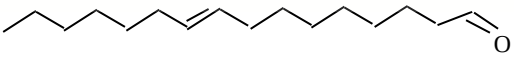
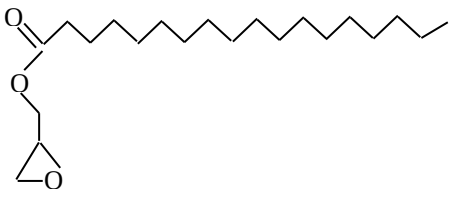
S/N	Metabolite	Formula	Structure	Relative Proportion (%)
1.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		1.41
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$		9.57
3.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		4.34
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$		2.36
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$		44.96
6.	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$		24.58
7.	Hexadecanoic acid, 2-hydroxyl-1, 3-propanediyl ester	$C_{35}H_{68}O_5$		1.95
8.	9, 12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$		7.15
9.	Octadecanoic acid, oxiranylmethyl ester	$C_{21}H_{40}O_3$		2.83
10.	Hexadecanoic acid, 2, 3-dihydroxypropyl ester	$C_{19}H_{38}O_4$		0.85

The grape seed oil, an essential oil of the hop cone was present in this sample with a proportion of 7.15 % which is higher than the proportion of the grape seed oil in both isomerized hop extract and the hop leaf extract as shown in Table 7. There were also present of other constituents in this extract which were absent in imported hops that may possibly give hop characters to beer. Based on these observations, *A. indica* could be a substitute for hops in beer brewing.

Vernoniaa mygdalina

The extract of *V. amygdalina* contained the least number of metabolites having nine (9) metabolites only. These metabolites and their relative proportion are shown in Table 3. It is shown in the Table that 6 – octadecenoic acid had the highest proportion of 43.42 % just like it had in isomerized hop extract and hop leaf extract as shown in Table 7. Based on this alone, *V. amygdalina* could substitute imported hops in beer brewing. Incidentally, this sample did not contain the essential oil of hop, the grape seed oil (9, 12-octadecadienoic acid). If this metabolite is not neglected, then *V. amygdalina* cannot substitute hops in beer brewing; although this extract contained other constituents common to the isomerized hop and hop leaf such as 11-octadecanoic acid methyl ester ($C_{19}H_{36}O_2$), octadecanoic acid methyl ester ($C_{19}H_{38}O_2$) and hexadecanoic acid ($C_{16}H_{32}O_2$) as presented in Table 7.

Table 3: Relative Proportion of Metabolites of *V. amygdalina*

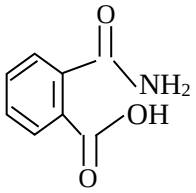
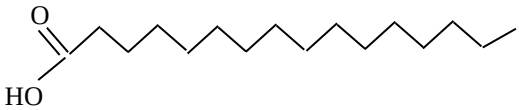
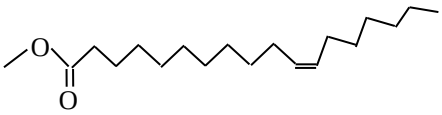
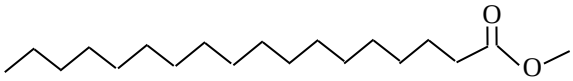
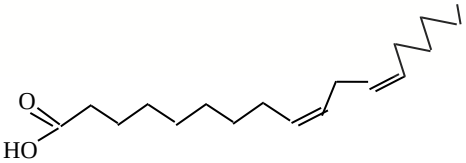
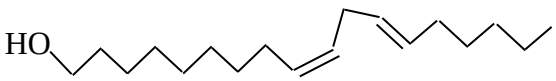
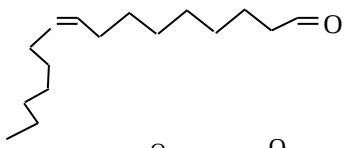
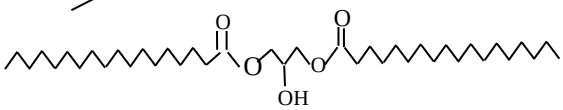
S/N	Metabolite	Formula	Structure	Relative Proportion (%)
1.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		1.46
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$		9.23
3.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		5.12
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$		2.63
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$		43.42
6.	Octadecanoic acid	$C_{18}H_{36}O_2$		24.06
7.	Hexadecanoic acid, 2-hydroxyl-1, 3-propanediyl ester	$C_{35}H_{68}O_5$		2.25
8.	9-Hexadecenal	$C_{16}H_{30}O$		8.31
9.	Octadecanoic acid, oxiranyl methyl ester	$C_{21}H_{40}O_3$		3.51

All these metabolites may contribute to the hop character of beer when *V. amygdalina* is used in lieu of imported hops.

Gongronema latifolium

From this work, it is shown that the extract of *Gongronema latifolium* contained 18 metabolites. These metabolites and their relative proportion are shown in Table 4. This extract contained both 6-octadecenoic acid and 9,12-octadecadienoic acid (grape seed oil) in proportions of 44.6 % and 7.95 % respectively. The Table shows that this extract contained other constituents such as hexadecanoic acid, 11-octadecenoic acid methyl ester, octadecanoic acid methyl ester, and octadecanoic acid 2-hydroxyl-1, 3-propanediyl ester ($C_{39}H_{76}O_5$). Extracts of imported hops also contained the aforementioned metabolites (Table 7).

Table 4: Relative Proportion of Metabolites of *G. latifolium*

S/N	Metabolite	Formula	Structure	Relative Proportion (%)
1.	Benzoic acid, 2-(aminocarbonyl)	$C_8H_7NO_3$		2.89
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$		10.69
3.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		0.77
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$		0.65
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$		44.61
6.	Octadecanoic acid, 2-(2-hydroxyl-ethoxy) ethyl ester (*Aquacera*)	$C_{22}H_{44}O_2$		25.46
7.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$		7.95
8.	9,12-Octadecadien-1-ol	$C_{18}H_{34}O$		4.91
9.	9-Hexadecenal	$C_{16}H_{30}O$		1.46
10.	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{16}O_5$		0.61

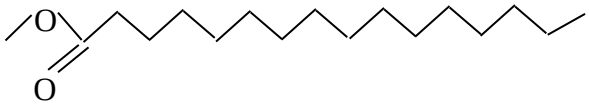
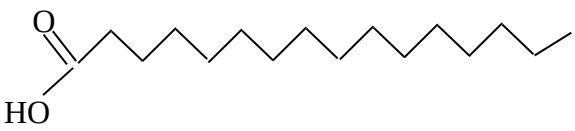
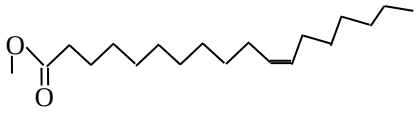
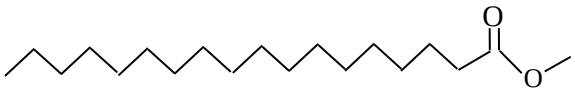
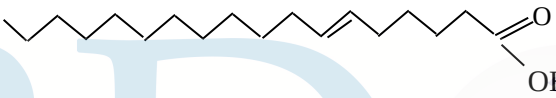
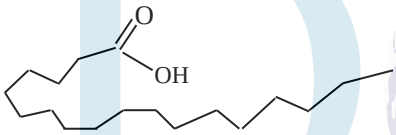
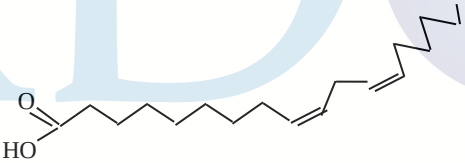
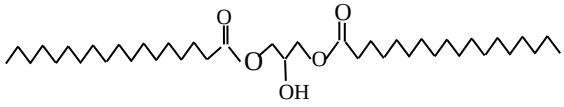
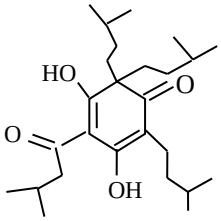
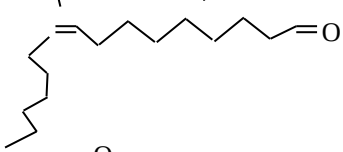
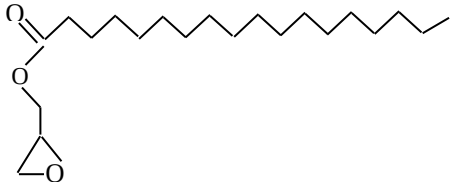
Based on these observations, *G. latifolium* could be a possible substitute to imported hops in beer brewing. Although the derivative of the alpha acid (dehydrocohumulunic acid) was conspicuously absent in this sample as sown in Table 7, there are other metabolites which were present in *G. latifolium* but were absent in imported hops. Such metabolites include benzoic acid-2-(amino carbonyl), and octadecanoic acid, 2-(2-hydroxylethoxy) ethyl ester, *aqua cera*.

Hop leaf

This sample contained eleven metabolites with 6 – octadecenoic acid having the highest proportion of 43.55 % and hexadecanoic acid, methyl ester having the least proportion of 1.13 %.

Table 5 shows these metabolites and their relative proportion. There was presence of lupulone (beta-lupulic acid) in hop leaf extract. Lupulone is a β-acid which contributes marginally in bitterness of beer. Also present in this sample was the grape seed oil. This is an essential oil of the hop cone responsible for flavour and aroma enhancement in finished beer. All the other metabolites present in this extract may contribute to other hop characters in beer.

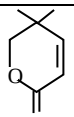
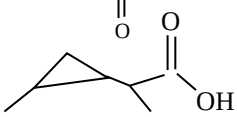
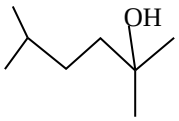
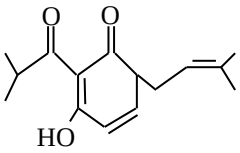
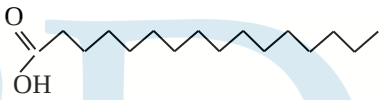
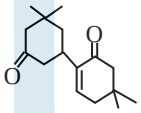
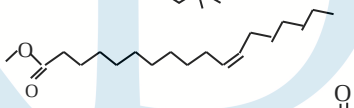
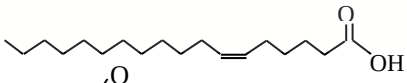
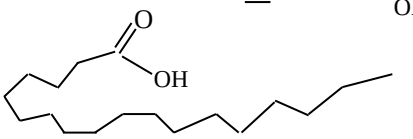
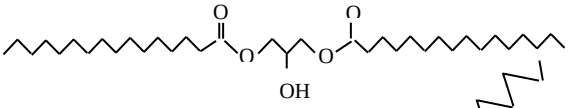
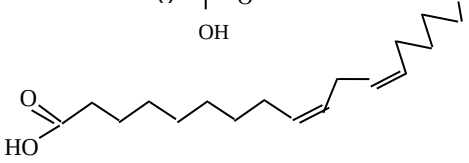
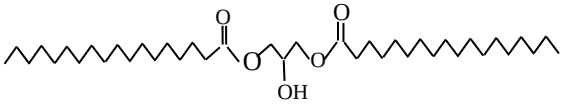
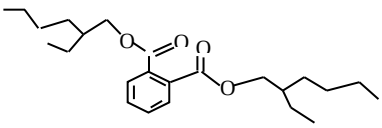
Table 5: Relative Proportion of Metabolites of Hop leaf

S/N	Metabolite	Formula	Structure	Relative Proportion (%)
1.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$		1.13
2.	Hexadecanoic acid	$C_{16}H_{32}O_2$		9.54
3.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		3.14
4.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$		2.45
5.	6-Octadecenoic acid	$C_{18}H_{34}O_2$		43.55
6.	Octadecanoic acid	$C_{18}H_{36}O_2$		25.56
7.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$		1.26
8.	Octadecanoic acid, 2-hydroxyl-1,3-propanediyl ester	$C_{39}H_{76}O_5$		1.63
9.	Lupulone (beta-lupulic acid)	$C_{26}H_{38}O_4$		2.02
10.	9-Hexadecenal	$C_{16}H_{30}O$		5.59
11.	Octadecanoic acid, oxiranyl, methyl ester	$C_{21}H_{40}O_3$		4.13

Isomerized hop

Isomerized hop extract contained fourteen metabolites with 6-octadecenoic acid having the highest proportion of 28.92%. The metabolites and their relative proportion are shown in Table 6.

Table 6: Relative Proportion of Metabolites of Isomerized hop

S/N	Metabolite	Formula	Structure	Relative Proportion (%)
1.	4,4-Dimethyl-2-buten-4-olide	$C_6H_8O_2$		3.62
2.	1,2-Dimethl cyclopropane carboxylic acid	$C_6H_{10}O_2$		9.90
3.	2,5-Dimethyl-2-hexanol	$C_8H_{18}O$		2.68
4.	Dehydro-cohumulinic acid	$C_{14}H_{18}O_3$		5.33
5.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		7.84
6.	4,4,5,5-Tetramethyl-bicyclo-hexyl-6-ene-2,3 dione	$C_{16}H_{24}O_2$		9.25
7.	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$		3.69
8.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$		1.21
9.	6-Octadecenoic acid	$C_{18}H_{34}O_2$		28.96
10.	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$		17.92
11.	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{35}H_{68}O_5$		1.24
12.	9,12-Octadecadienoic acid (Grape seed oil)	$C_{18}H_{32}O_2$		4.65
13.	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$		2.57
14.	1,2-Benzendicarboxylic acid, bis (2-ethylhexyl) ester	$C_{24}H_{38}O_4$		1.14

There was presence of dehydro-cohumulunic acid, a derivative of an alpha acid called cohumulone. Cohumulone generates iso-cohumulone by isomerization. Iso-cohumulones are chemical compounds that contribute to the bitter taste of beer and are in the class of compounds known as iso-alpha acids which contain approximately 40-80% bitter principles in the hop resin. The hop resin is known for its characteristic bitter taste in beer (Ajebesone and Aina, 2004).

There was also the presence of 9,12-octadecadienoic acid, the grape seed oil, which is an essential oil of the female hop cone responsible for flavouring and aroma enhancement in beer. All the other metabolites are however responsible for other characters of hop e.g. pronounced bacteriostatic activity that inhibits the growth of gram-positive bacteria in the finished beer and precipitation of proteins.

Metabolites Comparison of all the Extracts

Table 7 shows metabolites of the extracts from Nigerian plants in comparison with extracts of isomerized hop and hop leaf.

It is evident from Table 7 that 4,4-dimethyl-2-buten-4-olide ($C_6H_8O_2$); 1,2-dimethyl cyclopropane carboxylic acid ($C_6H_{10}O_2$); 2,5-dimethyl-2-hexanol ($C_8H_{18}O$); 4,4,5,5-tetramethyl bicyclo hexyl-6-ene-2,3,-dione ($C_{16}H_{24}O_2$); 1,2-benzen dicarboxylic acid bis (2-ethyl hexyl) ester ($C_{24}H_{38}O_4$) and dehydro-cohumulunic acid ($C_{14}H_{18}O_3$) are present in isomerized hop extract only.

It is also observed that hop leaf extract only contained lupulone ($C_{26}H_{30}O_4$), a β-acid known as beta-lupulic acid and octadecanoic acid oxiranyl methyl ester ($C_{21}H_{46}O_3$). All the extracts contained hexadecanoic acid ($C_{16}H_{32}O_2$), octadecenoic acid methyl ester ($C_{19}H_{36}O_2$), octadecanoic acid methyl ester ($C_{19}H_{38}O_2$) and 6-octadecenoic acid ($C_{18}H_{34}O_2$).

The extracts of hop leaf, isomerized hop, *G. kola*, *A. indica* and *V. amygdalina* contained octadecanoic acid ($C_{18}H_{36}O_2$) in common while the extracts of isomerized

hop, hop leaf, *G. kola*, *A. indica* and *G. latifolium* contained 9, 12-octadecadienoic acid, the grape seed oil ($C_{18}H_{32}O_2$) in common. The extracts of isomerized hop, hop leaf, *G. kola*, and *G. latifolium* only contained octadecanoic acid, 2-hydroxyl-1, 3-propandiyl ester ($C_{39}H_{76}O_5$).

Another significant observation is that each of the extracts of hop leaf, *G. kola*, *V. amygdalina* and *G. latifolium* contained 9-hexadecenal ($C_{16}H_{30}O$) which was absent in extracts of isomerized hop and *A. indica.* Also, each of the extracts of hop leaf, *G. kola*, *A. indica* and *V. amygdalina* contained hexadecanoic acid methyl ester ($C_{17}H_{34}O_2$). Hexadecanoic acid methyl ester was not present in both the extracts of isomerized hop and *G. latifolium*.

However, there are metabolites which were present in the local substitutes that were conspicuously absent in imported hops even though the Nigerian plants contained these metabolites differently, e.g. while *G. kola* alone contained 2-methyl-3, 13-octadecadien-1-ol ($C_{19}H_{36}O$), *G. latifolium*a lone contained octadecanoic acid -2-(2-hydroxyethoxy) ethylester ($C_{22}H_{44}O_2$) which is *aqua cera* and 9, 12-octadecadiene-1-ol ($C_{18}H_{34}O$). All these metabolites were completely absent in imported hops. This minor differences and major similarities in the constitution of metabolites in the local plants and those of imported hops is in agreement with the observation of Shellie *et al.* (2009), in their varietal characterization of hop by GC-MS analysis of hop cone extracts. These discrepancies observed in metabolites constitution of extracts from imported hops and those of Nigerian plants may explain the reason why the organoleptic character of beers brewed with imported hops and that of beers brewed with *G. latifolium* by Okafor and Anichie (1983) were more pronounced while their chemical properties did not differ much.

Table 7 Metabolites comparison of all the Extracts

S/N	Metabolte	Isomerized hop	Hop leaf	*G. kola*	*A. indica*	*V. amygdalina*	*G.latifolium*
		Relative Proportion (%)					
1.	4,4-dimethyl-2-buten-4-olide	3.62	-	-	-	-	-
2.	1,2-dimethyl-cyclopropane carboxylic acid	9.90	-	-	-	-	-
3.	2,5-dimethyl-2-hexanol	2.68	-	-	-	-	-
4.	Dehydro-cohumulunic acid	5.33	-	-	-	-	-
5.	4,4,5,5,tetramethyl-bicyclo hexyl-6-ene-2,3-dione	9.25	-	-	-	-	-
6.	1,2-benzenedicarboxylic, bis (-2-ethylhexyl) ester	1.14	-	-	-	-	-
7.	Hexadecanoic acid	7.84	9.54	9.30	9.57	9.23	10.69
8.	Octadecenoic acid, methyl ester	3.69	3.14	2.84	4.34	5.12	0.77
9.	Octadecanoic acid, methyl ester	1.21	2.45	1.62	2.36	2.63	0.65
10.	6-octadecenoic acid	28.96	43.55	44.09	44.96	43.42	44.61
11.	Octadecanoic acid	17.92	25.56	23.31	24.58	24.06	-
12.	Hexadecanoic acid, 2-hydroxy -1,3-propanediyl ester	1.24	-	1.92	1.95	2.25	-
13.	9,12-octadecadienoic acid (grape seed oil)	4.65	1.26	1.04	7.15	-	7.95
14.	Octadecanoic acid, 2-hydroxyl -1,3-propanediyl ester	2.57	1.63	2.84	-	-	0.61
15.	Hexadecanoic acid, methyl ester	-	1.13	0.69	1.41	1.46	-
16.	Lupulon (beta-lupulic acid)	-	2.02	-	-	-	-
17.	Octadecanoic acid, oxiranyl methyl ester	-	4.13	-	2.83	3.51	-
18.	9-hexadecenal	-	5.59	7.07	-	8.31	1.46
19.	2-methyl-3,13-octadecadienol	-	-	4.23	-	-	-
20	Octadecanoic acid, 2(-2-hydroxyethoxy) ethylester	-	-	-	-	-	25.46
21	9,12-octadecadien-1-ol	-	-	-	-	-	4.91
22.	Benzoic acid, 2(aminocarbonyl)	-	-	-	-	-	2.89
23.	Hexadecanoic acid-2,3-dihydroxypropyl ester	-	-	1.04	0.85	-	-

Furthermore, another interesting observation is that the relative proportion of metabolites which were commonly present in all the extracts are comparatively similar; example, the relative proportion of 6-octadecenoic acid is highest in each extract.

Iso- alpha Acid

Iso- alpha acid content in all the samples ranged between 7.95 and 12.53ppm with isomerized hop extract having the highest iso- alpha acid of 12.53ppm and hop leaf extract, the lowest iso- alpha acid of 7.75ppm. Table 8 shows that iso- alpha acid in all the extracts were found to be comparable with those of hop extracts. The result of iso-alpha acid of *V. amygdalina* is in agreement with that obtained by Adama *et al*. (2011) in their investigation of bitter leaf as local substitute for hops in the Nigerian brewing industry.

Table 8: Iso-alpha Acid of the Extracts

Extract	Iso-alpha acid (ppm)
Isomerized hop	12.53
Hop leaf	9.75
G. kola	8.78
A. indica	10.12
V. amygdalina	9.44
G. latifolium	9.67

These results are consistent with the report of Ashurt (1971) that non-polar fat solvents are suitable for the bittering constituents in hops and that bitterness level in beers depends on the age and method of storage of hops used in brewing.

Essential Oil

From Table 9, it was observed that the results obtained for essential oil content in all the extracts of the Nigerian plants were virtually in the same range but especially lowest in *A. amygdalina* and comparably low in hop leaf extract. The essential oil content of the Nigerian plants except *A. amygdalina* fell within the range of hop oil content of 0.88-1.63 g/100g as reported by Hough *et al*. in 1982. The low content of oil in *A. amygdalina* is attributed to the absence of the metabolite, 9,12-

octadecadienoic acid- the grape seed oil in this plant species as shown in Table 7 above.

Table 9: Essential Oil of the Extracts

Extract	Essential oil (g/100g)
Hop leaf	8.95
G. kola	8.78
A. indica	10.12
V. amygdalina	5.44
G. latifolium	9.67

Statistical Results

Metabolites present in imported hops (control) and the Nigerian plants differed significantly. Imported hops contained seven constituents that were not found in the Nigerian plants. Four metabolites were found to be present in both hop leaves/processed female hop inflorescence and the Nigerian plants. There were also five metabolites which were present in the Nigerian plants but absent in imported hops. However, the brewing values of the extracts did not show any significant difference.

CONCLUSION

This study has therefore shown that the extracts from tested Nigerian plants could present suitable substitutes for hops in beer brewing without significant alteration of the brewing values. Consequently, academic activity in the area of mixtures/blends of plant species which mimic hop taste is highly recommended.

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